SCIENTIFIC ARTICLES:

PLASMA BETA-ENDORPHIN LEVELS IN ORAL SURGERY PATIENTS FOLLOWING DIAZEPAM, FENTANYL OR PLACEBO[1]

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SUMMARY

Plasma beta-endorphin, pain and anxiety were measured in patients before, during, and 1 and 3 hours following oral surgery. Diazepam and fentanyl blocked the stress induced increase in plasma beta-endorphin experienced by patients administered placebo. Moreover, intra-operative anxiety and post-operative pain appear to constitute independent and possibly equipotent stimuli for release of pituitary beta-endorphin in humans.

INTRODUCTION

The adaptive response to stress takes many forms. In 1936, Seyle demonstrated that a major physiologic response is activation of the pituitary-adrenal axis.[1] Evidence advanced by Seyle and others lead to the conclusion that stress evokes pituitary secretion of adrenocorticotropic (ACTH), which in turn stimulates the synthesis and release of adrenal steriods such as cortisol. This is a critical component of the adaptive response since patients with a non-functional pituitary-adrenal axis are unable to survive physical stress.[2] Subsequent investigators established that the prohormone for ACTH is pro-opiomelanocortin (POMC).[3] As the name implies, cleavage products of POMC include beta-endorphin, an endogenous opioid peptide, beta-lipotropin, whose sequence contains beta-endorphin, and ACTH.[3] Beta-endorphin (B-END), beta-lipotropin (B-LPH) and ACTH are secreted together in response to a variety of stressors in both animals and man.[4-6] Yet the contribution of pituitary B-END, if any, to the adaptive response to stress is unknown.

Several lines of evidence suggest that pituitary endorphins may modulate stress responses through three general mechanisms. Firstly, endorphins are implicated as regulatory hormones by modulation of the secretory activity of other endocrine glands.[7,8] Secondly, they may act as trophic hormones by directly altering target site activity.[9,10] Finally, several lines of evidence suggest endorphin participation in the modulation of behavioral responses to pain.[11,12]

Data from numerous studies suggest the presence of several physiologic systems which are activated under stressful conditions and are capable of suppressing pain. These have been classified into a minimum of four systems, including neural endorphin, neural non-endorphin, hormonal endorphin and hormonal non-endorphin.[13,14] The imprecision of these terms accurately reflects current levels of knowledge in this field (i.e., "neural non-endorphin" may comprise one or more, parallel or interactive, neural networks). Additionally, other endogenous opioid peptides, such as met-enkephalin or dynorphin, may also participate as part of a hormonal endorphin system. Thus pituitary B-END, if at all relevant, may be only one of several endogenous pain suppression systems. Moreover, since stressors differ in their ability to release pituitary B-END,[5,15,16] the relative participation of pituitary B-END, as compared to the other pain suppression systems, probably varies under different experimental conditions. Taken together, this information supports the general observation that not all stressors evoke a stress induced analgesia (SIA) and, that not all forms of SIA are mediated by pituitary B-END. With this observation in mind, the following reviews the evidence suggesting a functional role for pituitary B-END in suppressing pain in animals and man.

In animals, data has been accumulating for over twenty years suggesting a functional involvement of the pituitary in modulating nociception. One of the earliest observations was made in 1960 by Murray and Miller with their demonstration that pituitary extracts potentiate morphine analgesia.[17] Interestingly, when given intravenously to mice, rats or cats, B-END has been estimated to be from 2.7 to 100 times more potent than morphine as an analgesic.[11,12,23,24] These doses, however, represent supra-physiologic blood levels. One strategy to circumvent this problem is to test for analgesia under conditions which provoke release of physiologic levels of pituitary B-END. Several investigators noted that certain stressors, including immobilization, electrical shock

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and insulin induced hypoglycemia, evoked release of pituitary B-END.[5,18,19] Additional studies demonstrated that these stressors produced a SIA.[13,14,20-22] These studies merely demonstrate an association between these forms of SIA and pituitary B-END release. Determination of a functional relationship between these forms of SIA and pituitary release of B-END has been evaluated in studies using interventions of either pituitary or B-END activity.

Two major forms of pituitary intervention have been used to address this functional relationship. First, numerous reports demonstrate the hypophysectomy abolishes SIA in response to a variety of environmental stressors, including immobilization, footshock and insulin induced hypoglycemia,[13,20-22,25-29] though this is not seen for all stressors,[13,22] The effect of hypophysectomy on SIA is not due to a generalized motor deficit since it does not alter hot plate escape behavior in non-stressed rats.[25,29] Thus, removal of the pituitary blocks the ability of certain stressors to produce a behavioral analgesia. A second, and more selective, form of pituitary intervention involves modulation of the pituitary-adrenal axis. It has long been known that pituitary secretion of ACTH (and thus B-END) is under negative feedback control by adrenal corticosteroids. Thus, administration of dexamethasone, a synthetic cortiocosteriod which blocks pituitary secretion of ACTH and B-END, will also block these forms of SIA.[26,27,30-34] Moreover, adrenalectomy, which results in a compensatory hypersecretion of pituitary ACTH and B-END, potentiates SIA.[33,34] Thus, alteration in pituitary activity, and more specifically, modulation of pituitary secretion of ACTH and B-END, results in parallel changes in specific forms of SIA.

A second series of studies have evaluated the functional relationship between SIA and pituitary B-END by altering B-END activity. Data must be interpreted with caution since these interventions do not discriminate between hormonal endorphin or neural endorphin pain suppression systems. Thus, the following only reviews studies utilizing stressors whose SIA is blocked by hypophysectomy. Numerous studies demonstrate that naloxone, an opiate antagonist, reverses or attentuates these forms of SIA.[20,21,31,35-38] Moreover, others have reported that, similar to chronic opiate administration, chronic SIA produces a behavioral tolerance[39] and a cross-tolerance with morphine.[40,42] Thus, stressors capable of producing a pituitary dependent SIA, demonstrate properties suggesting the functional involvement of endogenous opioid peptides.

Taken together, these data from animal studies suggest that, following certain stressful stimuli, a resulting SIA has a hormonal endorphin contribution probably due to release of pituitary B-END. Parallel studies in humans are lacking in the most part, due to ethical constraints. A strategy which overcomes this limitation utilizes surgical stress as a stimulus for pituitary B-END release. For example, women undergoing labor have significantly elevated plasma levels of B-END, which peak about 1 hour prior to delivery.[42,43] The amount of pituitary B-END released was associated with the difficulty or duration of labor.[43] Additionally, this release was blocked in patients receiving meperidine.[43] In a second series of studies pituitary B-END release was evaluated in patients undergoing laparotomy under general anesthesia.[44,45] Again, surgical stress evoked release of pituitary B-END. This release was blocked by pre-operative administration of fentanyl.[45] Thus, surgical stress appears to be a significant stimulus for release of pituitary B-END in humans. Moreover, the degree of release appears to be a function of both the surgical stress (difficulty or duration) and concurrent medications (i.e. opiates).

This report is the preliminary study of an ongoing project aimed at delineating i) the functional significance of pituitary B-END in modulating nociception in man, ii) the interactions of B-END release with standard pharmacologic agents, and iii) the effects of anxiety and pain on B-END release. The oral surgery model was chosen since it provides a normal, relatively drug-free population capable of providing simultaneous plasma samples and subjective reports before, during, and following the course of surgery.

METHODS

Twelve informed volunteers who presented at the Dental Clinic of the National Institute of Dental Research (NIDR) with at least two impacted third molars and devoid of systematic disease were the subjects of this study. All surgeries were started at approximately 10:00 and completed by 11:00 a.m. This minimizes possible variation due to circadian rhythms. All drugs were administered double-blind.

Patients reported to the clinic at least one week prior to surgery for a baseline sample. An intravenous infusion was established in a vein in the antecubital fossa and, following a 30 minute recovery period, a blood sample was drawn and questionnaires administered. Blood samples were collected with EDTA (at 1.4 mg/ml) and centrifuged. The resulting plasma was frozen over dry ice and stored at $-80°C$. Questionnaires for perceived levels of pain and anxiety were reported on psychological indices previously verified at the NIDR.[46-48] This included the visual analog scale (VAS) for pain and anxiety and Spielberger's State-Trait Anxiety Index (STAI). The STAI X-2 scale was utilized pre-operatively to determine that the treatment groups did not differ significantly in trait anxiety, a factor which may influence endorphin secretion during surgical stress.

On the day of surgery, an intravenous infusion was re-established with a 30 minute recovery prior to the pre-operative sample. Patients were randomly assigned to one of 3 treatment groups and given either intravenous saline placebo, fentanyl 0.1 mg or diazepam 20 mg 5 minutes before start of surgery. All

groups then received 2% lidocaine with 1:100,000 ephinephrine for local anesthesia. Intra-op blood was withdrawn during surgery, with at least one tooth extracted, while questionnaires were given immediately after surgery was completed. Additional blood samples and questionnaires were taken 1 and 3 hours post-op.

Immunoreactive B-END (B-END-LI) was extracted from plasma and concentrated as previously described.[49] In brief, 6 ml plasma was layered over two C18 columns in series (Sep Pak, Waters Associates, Milford, MA), washed with 3 ml of 0.05% trifluoroacetic acid and eluted with 3 ml of 50% acetonitrile containing 0.05% trifuluroacetic acid. The organic phase was removed by rotary evaporation followed by lyophilization. Recoveries of B-END standard added to plasma averaged 80%.

B-END-LI was measured by radioimmunoassay (RIA) using previously characterized antibody and incubation conditions.[48,50] The antibody binds to the C-terminal portion of B-END and therefore equally recognizes B-LPH. The assay detects 10 pg B-END-LI per tube, providing a calculated detection limit of 6 pg B-END-LI/ml plasma. All samples in this study were analyzed in the same RIA without knowledge as to treatment allocation.

Statistical significance was determined by ANOVA followed by Student's t-test. Since patients had yet to be segregated into treatment allocations, the interaction of anticipatory anxiety and plasma B-END-LI were analyzed for the group as a whole. Data is presented as the mean ($\bar{x}$) ± standard error of the mean (s.e.). A difference was accepted as significant if the probability that it occured due to chance alone was less than 5 percent, ($p < .05$).

RESULTS

Measures of anticipatory anxiety and plasma B-END-LI are illustrated in Table 1. Anticipatory anxiety can be estimated by comparing anxiety reported immediately prior to surgery (pre-op) to levels reported at least one week earlier. The study population can be treated as a whole since these comparisons occur before randomization. The STAI X-2 scale is an index of the patient's anxiety trait. The mean of 29.5 ± 2.0 is less than Spielberger's reported value of 38.25 + 9.14 (based on 231 college women.)[51] This comparison, while not definitive, supports the hypothesis that the patient population did not have a greater than average anxiety trait. The STAI X-1 scale is a state measure of anxiety. It demonstrated a strong tendency to increase from baseline to pre-op (29.5 to 34.5, $p = .08$). The VAS scale for anxiety showed similar trends (4.2 to 13.2 $p = .07$). In spite of these strong trends, plasma levels of B-END-LI did not change from baseline to pre-op (17.9 to 17.1 pg/ml).

Fig. 1 presents intra-operative levels of anxiety, pain and plasma B-END-LI for each of the three

TABLE 1

INTERACTION OF ANTICIPATORY ANXIETY AND PLASMA B-END-LI

SAMPLE	n	STAI X-2	STAI X-1	VAS ANXIETY	B-END-LI (pg/ml)
BASELINE	12	29.5* +2.0	29.5 +2.1	4.2 +1.6	17.9 +4.5
PRE-OP	12	—	34.5 +3.3	13.2 +4.7	17.1 +3.1

* $\bar{x}$ + s.e.

treatment groups. The placebo group reported a significant increase in anxiety from pre-op to intra-op on the VAS (16.8 to 44.0). Conversely, patients given diazepam or fentanyl reported little change in anxiety. Intra-operative reports of pain remained low, due to local anesthesia, and did not significantly differ between groups. Contrary to the fentanyl or diazepam groups, plasma levels of B-END-LI increased significantly from pre-op to intra-op in patients given placebo (18.8 to 49.5 pg/ml). Moreover, these intra-operative levels were significantly greater than the fentanyl treated group ($p < .05$) and marginally differed from patients given diazepam ($p = .067$).

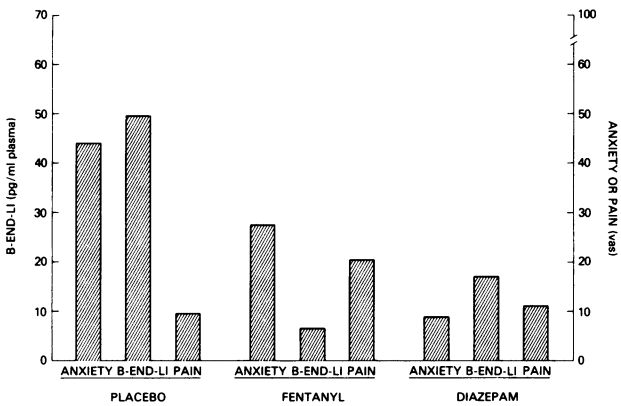

Fig. 1

1. Effects of placebo, fentanyl and diazepam on intra-operative levels of anxiety, B-END-LI and pain in patients undergoing oral surgery (n = 4/group)

Fig. 2 depicts levels of pain and plasma B-END-LI at the 1 hour post-operative period. Reported levels of anxiety were minimal in the post-operative period and are not presented in Figs. 2 and 3. The three treatment groups did not differ in pain reported 1 hour after surgery (Fig. 2); these values were similar to intra-operative levels (Fig. 1). At one hour post-op, plasma B-END-LI returned to intra-operative levels (Fig. 1). At one hour post-op, plasma B-END-LI returned to near pre-operative levels for the placebo group. Plasma levels of B-END-LI for the fentanyl and diazepam groups remained approximately the same at 1 hour as at previous pre-op and intra-op samples.

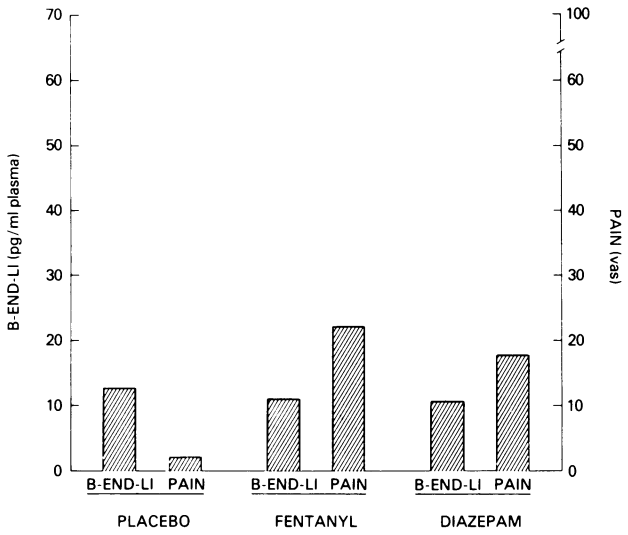

Fig. 2

2. One hour post-operative plasma levels of B-END-LI and pain for patients administered placebo, fentanyl or diazepam prior to oral surgery.

Fig. 3 illustrates the 3 hour post-operative data for reported pain and plasma B-END-LI levels. The onset of acute post-surgical pain is evident at this time. While levels of pain did not differ between groups, they were significantly greater than at any previous time. Similar to pain, plasma levels of B-END-LI increased dramatically for all groups.

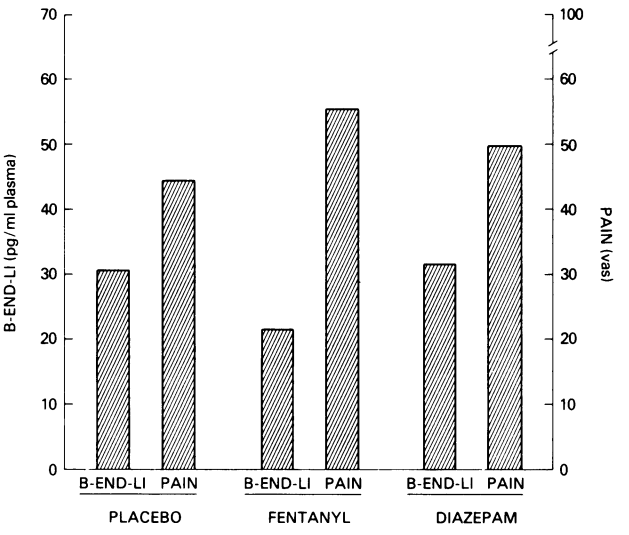

Fig. 3

3. Three hour post-operative plasma levels of B-END-LI and pain for patients administered placebo, fentanyl or diazepam prior or oral surgery.

DISCUSSION

The results of this study indicate that stress induced pituitary release of B-END, and its pharmacological manipulation, are measurable in the oral surgery model. Though these results are preliminary, due to sample size, several tentative conclusions can be reached. Earlier studies demonstrated that surgical stress or the stress accompanying labor can induce pituitary B-END release in humans.[42-45] This study confirms and extends these findings by demonstrating that both surgical stress and the onset of acute post-operative pain are sufficient stimuli to evoke pituitary release of B-END. Additionally, the present study confirms the observations of Dubois *et al.* and Thomas *et. al.,* that opiates block stress induced increases in plasma B-END in humans.[43,45] Opiate blockade of pituitary B-END release appears independent of its analgesic effects, since patients given either placebo of fentanyl reported similar levels of intra-operative pain (Fig. 1). This finding is in agreement opiate effects *in vitro* using human pituitary cultures,[52] yet is contrary to that generally observed in animals.[53] Thus, patients undergoing oral surgery demonstrate similar pituitary B-END responses to surgical stress and opiate administration as that reported for patients experiencing labor or laparotomy under general anesthesia.

The oral surgery model differs from other clinical models, however, by permitting simultaneous assessment of subjective responses throughout the surgical experience. In the pre-operative phase, moderate levels of anticipatory anxiety, as compared to baseline values on a non-surgery day, were insufficient to evoke release of pituitary B-END (Table 1). However, further increases in anxiety, under conditions of surgical stress, were associated with a marked increase in plasma B-END-LI (Fig. 1, Placebo vs. Diazepam). Pain is excluded as a possible contributing factor since it did not differ between the diazepam or placebo treated groups. One possible explanation of these findings requires anxiety to exceed a threshold level prior to stimulation of the pituitary adrenal axis. Alternatively, anticipatory anxiety measured pre-operatively may have been present for several days, with development of a physiologic habituation to this subjective state. In the post-operative phase, the onset of acute pain is associated with at least a two-fold increase in plasma levels of B-END for all three treatment groups. Anxiety can be excluded from consideration, since post-operative levels were minimal. Together, these data indicate that pain and anxiety constitute independent, and possibly equipotent, stimuli for release of pituitary B-END in humans.

Determination of the physiological significance of pituitary B-END in mediating adaptive responses to stress requires a multidisciplinary approach. In animal studies, several lines of evidence suggest that at least some forms of SIA require pituitary B-END release. Yet the relevance of these findings to humans is unknown. To date, clinical models capable of ethically evaluating the interrelations between stress, B-END release and pain, have been limited by employing a population with either altered endocrine

physiology or a population under general anesthesia. The oral surgery model circumvents these considerations. In addition to allowing simultaneous assessment of subjective and neuroendocrine components to surgical stress, this model permits many of the interventions employed in animal models. Thus, alterations of pituitary B-END release or B-END function while using the oral surgery model may prove to be a useful strategy in assessing the functional significance of pituitary B-END in humans in mediating adaptive responses to stress.

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